Influence of *Cyclooxygenase-2* (*COX-2*) Gene Promoter Polymorphism at Position −765 on Skin Cancer after Renal Transplantation

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TO THE EDITOR

Skin cancer is the most prevalent cancer in humans, and its incidence is 4–20 times higher in renal transplant recipients (RTRs; Grulich *et al.*, 2007) than in immunocompetent subjects, reaching an estimated incidence of 40–75% 20 years after transplantation (Moloney *et al.*, 2006). Hyperexpression of *cyclooxygenase 2* (*COX-2*) catalyzes the first step in the synthesis of prostaglandins, which may then function as tumor promoters or enhance initiation as oxidants (Rundhaug and Fischer, 2008). Furthermore, prostaglandin E2 (PGE2) induces *FOXP3* gene expression and T regulatory cell function in human $CD4^+$ T cells (Baratelli *et al.*, 2005), which may contribute to the immune escape of UV-mutated clones and ultimately lead to skin cancer (Beissert and Schwarz, 2009). A frequently occurring functional G→C polymorphism has been identified in the human *COX-2* gene promoter at position −765, with the C allele leading to a decreased promoter activity with low PGE2 production (Papafili *et al.*, 2002; Müller-Decker and Fürstenberger, 2007).

We conducted a study to investigate the association of the *COX-2* gene promoter polymorphism at position −765 with skin cancers in RTRs. Our working hypothesis was that RTRs carrying the C allele and consequently exhibiting reduced PGE2 production could experience less skin cancer due to the loss of the PGE2-promoting activity.

STUDY DESIGN AND POPULATION

Two cohorts of RTRs were investigated, including 365 patients from Besançon and 238 patients from Paris. The study design was approved by the research ethics committee of Besançon University Hospital, and all patients provided written informed consent before enrollment. The study adhered to the Declaration of Helsinki protocols. Skin cancer history was analyzed as a covariate.

DNA EXTRACTION AND ANALYSIS OF *COX-2* GENE PROMOTER POLYMORPHISM AT POSITION −765 (G→C)

Genomic DNA (gDNA) was obtained from peripheral blood leukocytes. Analysis of the *COX-2* gene promoter loci was done using a PCR-based genotyping assay (Papafili *et al.*, 2002; Courivaud *et al.*, 2009). Primer sequences used were 5′-CCGCTTCCTTTGTCCATCAG-3′ and 5′-GGCTGTATATCTGCTCTATATGC-3′. After PCR amplification of the polymorphism regions of interest (306 bp in length), the products were digested overnight with AciI restriction endonuclease (New England Biolabs, Beverly, CA). After separation by a 2% standard agarose gel electrophoresis, the digested PCR products of homozygous wild-type DNA were 188 and 118 bp in length (GG). Bands of three sizes (digested and undigested) were present in heterozygous DNA (GC). One band size (undigested) was present in muted homozygous DNA (CC). For each PCR, a negative control (PCR amplification without gDNA) was included. The functional characterization of this *COX-2* gene promoter polymorphism was validated in 62 RTRs in a previous study (Courivaud *et al.*, 2009) by measuring circulating PGE2 levels using a highly sensitive ELISA kit (R&D Systems, Lille, France).

STATISTICAL ANALYSIS

For normally distributed variables, C carriers and GG carriers were compared using the χ^2 test for dichotomic variables and Student's *t* test for continuous variables. Hardy–Weinberg equilibrium was assessed for the genotype distribution.

STUDY POPULATION

Three hundred and sixty-five patients from Besançon (cohort 1) were followed up for a mean duration of 9.5 ± 10 years after transplantation. The mean age at transplantation was 47 ± 13.5 years, and 228 RTRs (62.5%) were men. Skin cancers occurred in 28 patients (7.7%), which included 11 cases of squamous cell carcinoma (SCC), 13 cases of basal cell carcinoma (BCC), 3 cases of both SCC and BCC, and 1 case of melanoma. Two hundred and thirty-eight patients from Paris (cohort 2) were followed up for a mean duration of 8.5 ± 3.7 years after transplantation. The mean age at transplantation was 45 ± 12.5 years, and 134 (56.3%) were men. Skin cancer occurred in 16 patients (6.7%), which included 5 cases of SCC, 7 cases of BCC, 3 cases of both SCC and BCC, and 1 case of melanoma.

RELATION BETWEEN THE *COX-2* GENE PROMOTER POLYMORPHISM AND SKIN CANCER

Briefly, there was no difference in the distribution of the three genotypes in the two cohorts (Table 1). The observed allele frequencies were in Hardy–Weinberg equilibrium. In cohort 1, among the RTRs with skin cancer, 12 patients (9.6%) were C carriers and 16/240 (6.7%) were GG patients. In cohort 2,

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; COX-2, cyclooxygenase 2*; gDNA, genomic DNA; HR, hazard ratio; PGE2, prostaglandin E2; RTR, renal transplant recipient; SCC, squamous cell carcinoma*

Table 1. Genotype distribution of COX-2 polymorphism at position −765 in RTR patients

Patients	CC and CG (%)	GG (%)
Cohort 1 (Besançon)	125 (34.2)	240 (65.7)
No skin cancer	113 (90.4)	224 (93.3)
Skin cancer	12 (9.6)	16 (6.7)
Cohort 2 (Paris)	74 (31)	164 (69)
No skin cancer	69 (93.2)	153 (93.3)
Skin cancer	5 (6.7)	11 (6.7)
Cohorts 1 and 2	199 (33)	404 (67)
No skin cancer	182 (91.4)	377 (93.3)
Skin cancer	17 (8.5)	27 (6.7)

among the RTRs with skin cancer, 5 patients (6.7%) were C carriers and 11 (6.7%) were GG patients. Statistical analysis showed no difference between the genotypic distribution of RTRs presenting with skin cancer and the genotypic distribution of those without a history of skin cancer. In univariate analysis, only age at transplantation ($P=0.008$ and $P=0.021$, respectively) was associated with skin cancer. Cox regression analysis revealed that age at transplantation (hazard ratio (HR) 1.51; 95% confidence interval (CI) 1.33–2.45 and HR 1.39; 95% CI 1.12–1.85) was the only independent risk factor for skin cancer ($P=0.011$ and $P=0.034$, respectively).

CONCLUSION

We did not observe any significant difference in the genotype distribution of *COX-2* gene promoter polymorphism in RTRs between those with and those without skin cancer. Both C and GG carriers were predominant in RTRs without a history of skin cancer and, conversely, less common among RTRs with skin cancer. We previously demonstrated that C carriers have lower serum PGE2 levels in a transplantation setting (Courivaud *et al.*, 2009).

Our study does not confirm previous results, suggesting the protective effect of the *COX-2* gene promoter polymorphism at position −765 for BCC in RTRs (Lira *et al.*, 2007). However, this protective effect showed significance only in a subgroup of individuals (BCC patients who underwent transplantation before 50 years of age).

The discrepancy with our data might be explained by several hypotheses. First, we cannot exclude a limited statistical power due to the small number of BCC cases in our study (5 BCC cases among 26 patients <50 years of age). This is a significant concern because our statistical power calculation indicated that we would require at least 5 times this number of cases to detect a true effect size of ⩽2.0. For the difference observed in our study (GG versus CC and CG), this demonstration would require two cohorts comprising 6,322 RTRs—i.e., 12,644 RTRs—to demonstrate a risk below 2. Second, the effects of polymorphisms can vary from tissue to tissue, especially for inducible genes such as *COX-2* (Szczeklik *et al.*, 2004), through the loss of an Sp1 transcription binding site induced by the −765C variant along with the creation of a binding site for E2F, which is a cyclin-dependent regulator of expression of several genes (Papafili *et al.*, 2002). Third, the potentially protective effect of the −765C allele may be abolished by the additive effects of other risk factors, such as human papillomavirus infection and UV exposure. Indeed, *COX-2* −765C gene promoter polymorphism was associated with an increased risk of colorectal cancer (Xing *et al.*, 2008) or peptic ulcer disease (Saxena *et al.*, 2008) only in the presence of smoking and a high body mass index or *Helicobacter pylori* infection, respectively.

In conclusion, despite the weak statistical power, our data suggest that the COX-2 genotype does not represent a major risk factor for skin cancer in RTRs. However, given our small case numbers, we cannot exclude the possibility that this genotype may have a minor role in a subgroup of patients.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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François Aubin[1,2], Cécile Courivaud[3,4], Jamal Bamoulid[3,4], Alexandre Loupy[5], Marina Deschamps[3,6], Christophe Ferrand[3,6], Delphine Le Corre[7,8], Pierre Tiberghien[3,6,9], Jean-Marc Chalopin[3,4,9], Christophe Legendre[5], Eric Thervet[5], Philippe Humbert[2,3], Philippe Saas[3,6] and Didier Ducloux[3,4,9]

[1]Université de Franche Comté, EA3181, IFR133, Besançon, France; [2]Service de Dermatologie, Centre Hospitalier Universitaire de Besançon, Besançon, France; [3]INSERM UMR645, Université de Franche-Comté IFR 133, Etablissement Français du Sang Bourgogne Franche-Comté, Besançon, France; [4]Dialyse et Transplantation Rénale, Service de Néphrologie, Centre Hospitalier Universitaire de Besançon, Besançon, France; [5]Service de Transplantation Rénale, Centre Hospitalier Universitaire Necker, Paris, France; [6]Plateforme de Biomonitoring, Etablissement Français du Sang Bourgogne Franche-Comté, Besançon, France; [7]Université Paris Descartes, Paris, France; [8]INSERM UMR-S 775 Centre Universitaire des Saints-Pères, Paris, France and [9]Centre Hospitalier Universitaire de Besançon, Besançon, France
E-mail: francois.aubin@univ-fcomte.fr

REFERENCES

Baratelli F, Lin Y, Zhu L *et al.* (2005) Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. *J Immunol* 175:1483–90

Beissert S, Schwarz T (2009) Ultraviolet-induced immunosuppression: implications for photocarcinogenesis. *Cancer Treat Res* 146:109–21

Courivaud C, Bamoulid J, Loupy A *et al.* (2009) Influence of *cyclooxygenase-2 (COX-2)* gene promoter polymorphism -765 on graft loss after renal transplantation. *Am J Transplant* 9:2752–7

Grulich AE, van Leeuwen MT, Falster MO *et al.* (2007) Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 370:59–67

Lira MG, Mazzola S, Tessari G *et al.* (2007) Association of functional gene variants in the regulatory regions of COX-2 gene (PTGS2) with nonmelanoma skin cancer after organ transplantation. *Br J Dermatol* 157:49–57

Moloney FJ, Comber H, O'Lorcain P *et al.* (2006) A population-based study of skin cancer incidence and prevalence in renal transplant recipients. *Br J Dermatol* 154:498–504

Müller-Decker K, Fürstenberger G (2007) The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. *Mol Carcinog* 46:705–10

Papafili A, Hill MR, Brull DJ *et al.* (2002) Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 22:1631–6

Rundhaug JE, Fischer SM (2008) Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. *Photochem Photobiol* 84:322–9

Saxena A, Prasad KN, Ghoshal UC *et al.* (2008) Polymorphism of -765G > C COX-2 is a risk factor for gastric adenocarcinoma and peptic ulcer disease in addition to H pylori infection: a study from northern India. *World J Gastroenterol* 14:1498–503

Szczeklik W, Sanak M, Szczeklik A (2004) Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. *J Allergy Clin Immunol* 114:248–53

Xing LL, Wang ZN, Jiang L *et al.* (2008) Cyclooxygenase 2 polymorphism and colorectal cancer: −765G>C variant modifies risk associated with smoking and body mass index. *World J Gastroenterol* 14:1785–9

IL-17A Has an Important Role in the Acute Attacks of Behçet's Disease

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TO THE EDITOR

Behçet's disease (BD) is a chronic, relapsing, systemic vasculitis of unknown etiology. Patients with BD exhibit elevated levels of proinflammatory cytokines and the affected organs show a significant neutrophile and lymphocyte infiltration. Current evidence suggest that the activated lymphocytes contribute to neutrophile and endothelial cell activation in these patients (Akman *et al.*, 2007; Alpsoy *et al.*, 2007). Recently, a new subset of Th cells, Th17 cells, in part characterized by their production of IL-17 has been identified and their roles in inflammation or immune regulation are under intensive investigation. IL-17 promotes inflammation by inducing various proinflammatory cytokines and chemokines; it modulates the secretion of IL-1, tumor necrosis factor-α, IL-6, IL-8, and prostaglandin E2. IL-17 also increases CXC chemokine secretion. Therefore, IL-17 causes neutrophil influx and regulates neutrophil-mediated inflammatory responses. Th17 cells have the most remarkable effects on neutrophil activity within T-cell sub-populations (Furuzawa-Carballeda *et al.*, 2007). It is well known that neutrophil activity is increased in BD. Again, IL-6, IL-1, and tumor necrosis factor-α, which have a role in the differentiation of naive T cells to Th17 cells, are found to be elevated in patients with BD (Fossiez *et al.*, 1996; Akman *et al.*, 2006). Serum G-CSF level was also reported significantly higher in active patients than in those inactive patients with BD (Kawakami *et al.*, 2004).

In a recent study, Chi *et al.* (2008) have shown elevated production of IL-17, IL-23, and IFN-γ by peripheral blood mononuclear cells (PBMC) besides increased frequencies of IL-17 and IFN-γ-producing T cells in BD patients with active uveitis. In this study, we particularly aimed at investigating the role of IL-17A in the activity and different organ involvements of the disease.

Forty-five patients (24 women, 21 men; mean age, 40 years) with BD, diagnosed according to the criteria of the International Study Group for BD (1990), and 33 age- and sex-matched healthy volunteers (17 women, 16 men; mean age, 36 years) were enrolled in the study. The study was approved by the Ethics Committee of Akdeniz University (Institutional approval number: 2007.02.0122007). Informed consent was obtained from all participants, and study was conducted according to the Declaration of Helsinki Principles. Control group had neither family history nor symptoms related to BD. Twenty-two patients (10 women, 12 men, mean age 41) with BD were at inactive stage, whereas 23 of them (14 women, 9 men, mean age 39 years) were at active stage. Any patient having any active sign of the disease at the time of blood drawn was accepted at active stage. All patients had oral ulcers, and in 16 of them this finding was at active stage. The same figure was 16 and 2 for genital ulcers, 13 and 1 for erythema nodosum, 23 and 6 for articular involvement, 18 and 8 for ocular symptoms. In addition, six patients had vascular, and two gastrointestinal involvement.

We examined serum levels of IL-17A by ELISA. *In vitro* IL-17A response of PBMC of patients with BD and controls after stimulation with *Streptococcus sanguis* (*S. sanguis*), *Escherichia coli* (*E. coli*), and phytohemaglutinin were evaulated by enzyme-linked immunosorbent spot method. The proportion of IL-17A-secreting cells (Th17 cells) in nine patients with active organ involvement and nine healthy

Abbreviations: BD, Behçet's disease; PBMC, peripheral blood mononuclear cells